REMARKS

Claims 1-39 are canceled. Claims 40-69 are added, and now active in this application.

A. Rejections Based Upon Ostensible Prior Art Under 35 U.S.C. 102(b)

Claims 1-4, 6-23 and 37-39 stand rejected under 35 U.S.C. §102(b) as being anticipated by Sapolsky *et al.* (US 2003/0008292).

Claims 1-4, 6-23 and 37-39 stand rejected under 35 U.S.C. 102(b) as being anticipated by Jones (US 2002/0072055).

Claims 1-4, 6-23 and 37-39 stand rejected under 35 U.S.C. 102 (b) as being anticipated by Yu *et al.*(WO 02/34939).

Claims 1-4, 6-23 and 37-39 stand rejected under 35 U.S.C. 102(b) as being anticipated by Eijk *et al.* (WO 01/49882).

Claims 1-4, 6-23 and 37-39 stand rejected under 35 U.S.C. 102(b) as being anticipated by Kato (EP 0735144).

However, none of the above cited references, either alone or in combination, discloses nor suggests the claimed invention. In particular, none discloses or suggests either the claimed method for generating short DNA fragments or the short DNA fragments which are obtained by the claimed method.

The claimed method is based on the selective fragmentation of nucleic acids to be analyzed using a combination of two restrictions enzymes E1 and E2 and a double-stranded adapter AA' (see figures 1 to 8 of the present application).

- Step a) of the claimed method comprises generating DNA fragments F1 with blunt or cohesive ends using the restriction enzyme E1 which randomly fragments the sample of nucleic acids.

- Step b) comprises generating DNA fragments F'1 by ligation of the ends of the DNA fragments F1 with a complementary double-stranded adapter AA' which is chosen so that the junction of the 3'end of the adapter AA' and the 5' end of the fragments F1 which corresponds to the 3' end of the E1 enzyme restriction site, contains the first base pairs only but not the entire sequence of the recognition site of a restriction enzyme E2 whose cleavage site is located downstream of its recognition site (type IIs restriction enzyme); see for example figures 2, 4-1, 5-1 and 6-1 of the present application).

- Step c) comprises the selective cleavage of a sub-population of fragments F'1 whose F1 part (specific sequence from the nucleic acid sample) contains at its 5'end, the 3' end of the E2 enzyme recognition site, using the restriction enzyme E2 (see figures 4-2, 5-2 and 6-2 of the present application, thereby generating a fraction of short DNA fragments F2.

The short DNA fragments (F2) generated by the claimed method are unique (both with respect to their difference from the cited references, and, hence, their patentability) in that the specific sequence is flanked by the recognition and the cleavage site of the E2 enzyme and the 5' end of the specific sequence (F1 part) consists of the last base pairs of the E2 restriction enzyme restriction site.

None of the cited references describes or suggests the selective fragmentation of nucleic acids to be analyzed using a combination of two restrictions enzymes E1 and E2 (type IIS enzyme) and a double-stranded adapter AA' which is complementary to the E1 restriction site and is chosen so that the 3' end of the

E1 enzyme restriction site contains the first base pairs only but not the entire sequence of the E2 enzyme recognition site:

- Sapolsky *et al.* (US 2003/0008292) disclose (figures 2-3 and pages 2-9) a method for identifying sequence based nucleic acid markers comprising (paragraph 34, page 3):

  1. digesting nucleic acids with a first type IIS restriction enzyme (RE) having a recognition site on the nucleic acids sequence (EarI site: 5' CTCTTC3'), thereby cleaving the sequence downstream of its recognition site,

  2. ligating a first adapter which comprises a second type IIS RE recognition site to the cleaved sequence; the adapter comprises the complete sequence of the type IIS RE recognition site (HgaI site 5'GCGTC 3') as shown in figures 2-3,

  3. digesting the ligated sequence with the second type IIS RE that cleaves downstream of its recognition site and within the first type IIS RE recognition site (figures 2-3), thereby cleaving the sequence, and

  4. ligating the cleaved sequence with a second adapter sequence, thereby generating short DNA fragments having an ambiguous sequence (specific sequence from the nucleic acid sample) sandwiched between two different type IIS RE recognition sites (5' CTCTTCNnnnGCGTC 3'; figures 2 and 3 and page 3, middle of paragraph 34).

  The method disclosed by Sapolsky does not entail the digestion of a subset of fragments (selective fragmentation) since all the ligated fragments have the type IIS RE recognition site sequence.

- Yu *et al* (WO 02/34939) disclose (pages 5-6 and 11-15 and figure 2) a method of analyzing DNA comprising:

  1. ligating a first oligonucleotide (adapter; figure 2) and a DNA fragment (obtained by RE digestion) to form a ligated product. The adapter comprises a known sequence and a type IIS RE recognition site, *i.e.* the complete sequence of the type IIS RE recognition site (GsuI site: 5' CTGGAG 3') as shown in figure 1a, and

  2. digesting the ligated fraaments with the type IIS RE to release a second nucleotide comprising the first oligonucleotide and a sequence from the DNA fragment.

  The method disclosed by Yu *et al*. generates short DNA fragments having a specific sequence (sequence from the DNA fragment) flanked by the recognition and the cleavage site of a type IIS RE. However, the 5' end of the specific sequence does not contain the 3' end sequence of the type IIS RE recognition site because the complete sequence of the type IIS recognition site is in the adapter (figure 2).

  The method disclosed by Yu *et al*. does not entail the digestion of a subset of fragments (selective fragmentation) since all the ligated fragments have the type IIS RE recognition site sequence.

- Van Eijk *et al*. (WO 01/49882) disclose (pages -10, 24-25, 27-28 and figure 1) a method for generating an oligonucleotide (short DNA fragment) comprising:

  1. Providing a first dsDNA,

2. Ligating the first dsDNA to a second dsDNA comprising a type IIS recognition site (*i.e.* the complete sequence of the type IIS RE recognition site), so as to provide a ligated dsDNA, and

3. Restricting the ligated DNA with the type IIS RE so as to obtain a first and a second IIS-restricted ds DNA.

The method disclosed by Van Eijk *et al.* generates DNA fragments having specific sequence (sequence from the DNA fragment) flanked by the recognition and the cleavage site of the type IIS RE. However, the 5' end of the specific sequence does not contain the 3' end sequence of the type IIS RE recognition site because the complete sequence of the type IIS RE recognition site is in the adapter (figure 2).

The method disclosed by Van Eijk *et al*. does not entail the digestion of a subset of fragments (selective fragmentation) since all the ligated fragments have the type IIS RE recognition site sequence.

- Kato (EP 0735144) discloses (pages 5-6 and 11-13, figures 1-3) a method for molecular indexing of expressed genes using combination of type IIS restriction enzymes comprising:

1. digesting nucleic acids (cDNA) with a first type IIS restriction enzyme (RE),

2. ligating each of the resultant cDNA fragments to one from a pool of 64 biotinylated adaptors cohesive to all possible overhangs,

3. digesting the resultant cDNA fragments further with a second type IIS RE which is different from the first type IIS RE, thereby obtain a first cDNA sample.

The method disclosed by Kato does not entail the digestion of a subset of fragments (selective fragmentation) since all the fragments are ligated to the adaptor which has the type IIS RE recognition site sequence.
Kato does not use adaptors comprising type II recognition site. The type IIS recognition sites are in the DNA sequence (figures 1 and 3). The short DNA fragments generated by the method of Kato comprise a specific sequence flanked by two type IIS cleavage sites (figure 1; step (4) fragment (i)).

- Jones (US 2002/0072055) discloses (pages 5-16 and figures 1-4) a method for sequencing DNA comprising:

1. digesting an immobilized DNA molecule (template precursor) with a first type IIS restriction enzyme (RE) to generate a free fragment and a bound fragment,

2. ligating the free end of the bound fragment to a fluorescent adapter comprising a type IIS recognition site, *i.e.* the complete sequence of the type IIS RE recognition site (FokI site), three degenerate nucleotides with a fixed G, A, T or C 5' end

3. identifying the ligated adapter by flurometry

4. PCR amplify the ligated fragment

5. Bind the PCR product to a solid matrix

6. Repeat step 1.

The method disclosed by Jones does not entail the digestion of a subset of fragments (selective fragmentation) since the template precursor has the type IIS RE recognition site sequence.

Additionally, the method disclosed by Jones does not generate isolated short DNA fragments having a specific sequence (sequence from the DNA fragment) flanked by the recognition and the cleavage site of the type IIS RE because the method disclosed by Jones is a DNA sequencing method (figures 1-4).

Clearly, one skilled in the art would not have been put in possession of the claimed invention by any single cited reference or combination thereof.

Hence, each and all of these grounds of rejections are believed to be unsustainable and should be withdrawn.

B. Rejection Under 35 U.S.C. 103(a)

At the outset, Applicants clarify that the subject matter of the various claims was commonly owned at the time the invention covered by these claims was made.

Claim 5 stands rejected under 35 U.S.C.103(a) as being unpatentable over anyone of the previously-cited references above in view of Keith *et al*.

However, as noted above, none of the above-cited references would have put one skilled in the art in possession of the claimed invention.

Furthermore, Keith *et al*. teaches only a method of simultaneously preparing DNA fragments by restriction enzyme digest and ligating said fragments to another nucleic acid of desired function.

Keith *et al*. neither disclose nor suggest the elements missing from the above-cited other references, such as the selective fragmentation of nucleic acids to be analyzed using a combination of two restrictions enzymes E1 and E2 (type IIS enzyme) and a double-stranded adapter AA' which is complementary to the E1 restriction site and is chosen so that the 3' end of the E1 enzyme restriction site contains the first base pairs only but not the entire sequence of the E2 enzyme recognition site.

Thus, even the combination of all of Sapolsky *et al*. Jones, Yu *et al* and Van Eijk *et al* with Keith *et al* would have failed to provide one skilled in the art with sufficient guidance, motivation or enablement to have attained the claimed invention.

Hence, this ground of rejection is unsustainable and should be withdrawn.

C. Objection To The Specification

This objection is deemed moot in view of the attached Substitute Specification and mark-up copy attached to this response. See paragraph 3 of the Action of March 25, 2010.

D. Rejection Under 35 U.S.C. 101

Claim 21 stands rejected under 35 U.S.C. 101.

However, in view of the above amendments this ground of rejection is now moot.

E. Rejection Under 35 U.S.C. 112, second paragraph

Claims 1-23 and 37-39 stand rejected under 35 U.S.C. 112, second paragraph.

However, in view of the above amendments, this ground of rejection is moot, And, in particular, with respect to each and all of the objected terms and phrases noted at pages 5-8 of the Action of March 25, 2010.

F. Drawing Objections

The objection to the drawings at paragraph 2 (on page 3) of the Action of March 25, 2010, is deemed to be moot in view of the Formal Drawings submitted herewith. Specifically, Formal Drawings of Figs. 1-7 (13 sheets) are attached to this response. Figure 8 is urged as acceptable as is.

Accordingly, in view of all of the above amendments and remarks, and attachments to this response, it is believed that this application now stands in condition for allowance. Favorable consideration is earnestly solicited.

Respectfully submitted,

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